

*Application for*  
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**APPARATUS FOR MICROINJECTION OF SAMPLE  
INTO AMPHIBIAN OOCYTES**

## DESCRIPTION

### APPARATUS FOR MICROINJECTION OF SAMPLE INTO AMPHIBIAN OOCYTES

#### Background of the Invention

##### (1) Technical Field

The present invention relates to an automated apparatus for injecting a sample of gene, pigment, protein, peptide or drug into oocytes of Amphibia such as frog using a pipette-like needle. The present invention further pertains a method for injecting the sample of gene, pigment, protein, peptide or drug into the specified position of the amphibian oocytes, the amphibian oocytes with a guaranteed quality for injection of the sample, and a method for selecting or assigning the amphibian oocytes into which the sample is injected at a specified position and depth.

##### (2) Background Art

Since oocytes of frogs such as *Xenopus laevis*, into which gene, pigment, protein, peptide or drug is injected, have a comparatively large size and can be obtained with a low cost and in large quantities, they are widely used for the purpose of confirming actions of pigment, protein, peptide and drug on viable cells, analysis of gene functions, and production of proteins as a gene product. To this purpose, the individual research scientists breed frogs by themselves and collect the oocytes.

Heretofore, in the injection of a sample such as gene, pigment, protein, peptide and drug into the oocytes of Amphibia such as frog, technicians manually injected with a pipette-like needle in which these samples are packed, into the oocytes under a microscopic observation using a manipulator. The pipette is mounted on the injector and a constant amount of sample is injected and exhaled into cells by an action of an oil pressure or an air pressure. Further, JP-A-5-192171 and JP-A-6-343478 disclose a method for injection and exhalation of a constant amount of sample into cells by applying a voltage. These prior arts disclose techniques for injection of sample by approaching needle manually to the cell under observing the cell microscopically.

## Summary of the Invention

### (1) Problems to be solved by the invention

The above manual injection of samples such as genes had a problem that a rate of oocytes, into which sample was successfully injected, was varied depending on skills and experiences of technicians. This was due to a difference in numbers of oocytes, into which the sample could be injected within a fixed time, depending on the individual technician, and as a result, a degree of denaturation of the sample was varied in a time-dependent manner among the technicians. It is an object of the present invention to provide a method to give a constant numbers of cells per hour to be treated without skills and experiences of technicians.

Further, the above described prior art did not take the consideration for unifying an injection depth of samples into the oocytes at a constant depth. Consequently, it was difficult to control the depth even by a skilled technician. As a result, an injection of a sample into a specified cell organelle such as a nucleus had to depend on by chance. It is another object of the present invention to provide an easier method of an injection of sample into a cellular organelle such as a nucleus, in which an injection can be controlled in a depth direction by unifying an injection depth of the sample.

Furthermore, in the above described prior art, since no consideration was given for memorizing information of cells in the injection of sample, it was difficult to obtain a correlation between the information on the sample injection and the subsequent cell reaction. Consequently, it is further object of the present invention to provide a method for obtaining the correlation between them.

In addition, in the conventional techniques, the oocytes were prepared individually and a mass production or a production on demand in good timing were impossible.

Consequently, it is further more object of the present invention to provide the oocytes, into which a specified sample is injected, or the production, sale and transportation of the oocytes with a guaranteed injection of the specified sample at a

constant position.

(2) Means for solving the problems

In order to solve the above problems, the present invention provides an apparatus for automatically injecting the sample such as gene, pigment, protein, peptide or drug into any constant position and any constant depth of the oocytes of Amphibia such as frog by using a pipette-like needle.

Namely, the present invention provides an apparatus for microinjection of samples into amphibian oocytes comprising a tray for holding a plurality of the amphibian oocytes, an injection needle for injecting a sample into the said amphibian oocytes, a driving means for moving a relative position of the said tray to the said injection needle and a controlling means for controlling the said movement by inputting a depth of the said injection needle for the said tray or the said amphibian oocytes in the injection of the sample, and injecting the sample into the said amphibian oocytes at the said depth.

The present invention further provides a system for microinjection of samples into amphibian oocytes comprising a tray for holding a plurality of the amphibian oocytes, an injection needle for injecting sample into the said amphibian oocytes, a driving means for moving a relative position of the said tray to the said injection needle in a three dimensional direction, a controlling means for controlling the said movement, an information obtaining means for obtaining a visual information of the said amphibian oocytes in the microinjection, and a memorizing means for accumulating the said information, and injecting the said sample into the said amphibian oocytes.

As a result, the sample can be injected rapidly into the plurality of amphibian oocytes at an almost constant depth.

Further, the tray has a plurality of wells having a cylindrical structure with a flat base or with a conical base having a maximum diameter of 105 - 150% of a diameter of the amphibian oocytes. As a result, the sample can be injected into the identical surface in about 80% of oocytes on the tray without applying any special means.

We have found that when mRNA was injected into the oocytes, in case that mRNA was injected into the animal hemisphere of the oocyte or mRNA was injected into

the vegetal hemisphere, expression efficiency or the functional expression efficiency is different. Namely, in order to suppress a variation of the expression efficiency of the function of a protein in oocytes, it is important to collect the oocytes, in which mRNA is injected into the same hemispherical surface. In the present invention, it is possible to memorize information of cellular area in the injection of the sample and to induce easily a correlation between the information and the subsequent cell reaction.

Further, the present invention provides a method for automatic microinjection of samples into amphibian oocytes comprising,

using an apparatus having a tray for holding a plurality of the amphibian oocytes and an injection needle for injecting a sample into a plurality of the said amphibian oocytes,

a step for setting a depth of the said injection needle for the said tray or the said amphibian oocytes at the first depth,

a step for injecting the sample into the first oocyte in a plurality of the said amphibian oocytes using the said injection needle at the said first depth,

a step for automatically moving a relative position of the said tray to the said injection needle, and

a step for subsequently injecting the sample into the second oocyte in a plurality of the said amphibian oocytes by using the said injection needle to the said first depth.

Furthermore, the present invention provides a method for automatic microinjection of samples into amphibian oocytes comprising,

using an apparatus having a tray for holding a plurality of the amphibian oocytes and an injection needle for injecting a sample into a plurality of the said amphibian oocytes,

a step for injecting the sample into the first oocyte in a plurality of the said amphibian oocytes using the said injection needle,

a step for moving a relative position of the said tray to the said injection needle,

a step for subsequently injecting the sample into the second oocyte in a plurality of the said amphibian oocytes using the said injection needle,

a step for obtaining a condition of oocyte in the injection of the said sample as a visual information, and

a step for accumulating the said visual information.

According to the invention of the above described apparatus or method for injection of the sample into the amphibian oocytes, the present invention further provides a plurality of the amphibian oocytes wherein the sample is injected under a substantially equal condition in the injection depth of the sample.

The present invention also provides the said amphibian oocytes wherein the sample is further injected under a substantially same condition in the injection position of the sample.

In addition, the present invention provides the following methods.

A method for preparation of a group of the amphibian oocytes injected with a sample comprising,

using an apparatus having a tray for holding a plurality of the amphibian oocytes and an injection needle for injecting sample into a plurality of the said amphibian oocytes,

moving a relative position of the said injection needle to the said tray,

injecting the sample into each of a plurality of the said respective amphibian oocytes using the said injection needle,

obtaining each visual information of the said amphibian oocytes in the injection, and

collecting a plurality of the oocytes in which the sample is injected into the animal hemisphere of the oocyte, or a plurality of the oocytes in which the sample is injected into the vegetal hemisphere of the oocyte in a plurality of the said amphibian oocytes based on the said visual information.

A method for selling or assigning a plurality of the amphibian oocytes comprising selling or assigning as a set a plurality of the amphibian oocytes, into which the sample is injected under a substantially equal condition in the injection depth of the sample, and attaching the information on the injection of the sample into a plurality of the amphibian oocytes to the set.

In addition, a method for selecting or assigning a plurality of the amphibian oocytes comprising putting a plurality of the amphibian oocytes into which the sample is injected under a substantially equal condition in the injection depth of the sample into a vessel, and attaching a label on which the information on the injection of the sample into a plurality of the said amphibian oocytes is described to the said vessel.

In this connection, the information on the injection of the sample into a plurality of the amphibian oocytes relates to at least any one of a date and time of the injection, a term for guarantee of a quality, a position where the sample is injected, a depth at which the sample is injected and a probability of expression.

As a result, according to the present invention, the oocytes having substantially identical condition in relation to the injections as well as their information can be obtained.

#### Brief Description of the Drawings

Fig. 1: A construction of apparatus of the present invention.

Fig. 2: An illustrative example of a tray used in the present invention.

Fig. 3: An illustrative example of a vessel used in the present invention.

Fig. 4: A correlation between fluorescence of GFP injected into oocyte and a ligand response of histamine receptor expressed by co-injection.

Fig. 5: A correlation between an area of gene injection (animal hemisphere and vegetal hemisphere) and a ligand response.

Fig. 6: Use of oocyte as a histamine sensor.

Fig. 7: A method of transferring oocytes after injection of sample.

#### Explanation of symbols:

1: control unit, 2: auxiliary control unit, 3: monitor, 4: moving table for injection needle, 5: injection device, 6: injection needle, 7: CCD camera, 8: digital camera, 9: tray, 10: light source, 11: orthogonal moving table, 12: horizontal moving table, 13: oocytes, 14: physiological saline for Amphibia, 21: vessel, 22: label, 23: cold insulator, 31: histamine receptor gene, 32: histamine receptor, 33: sample containing histamine, 34: histamine

response (positive), 35: sample without histamine, 36: histamine response (negative), 41: syringe, 42: buffer solution for Amphibia, 43: antibiotics, 44: outer vessel, 45: package.

### Description of the Preferred Embodiment

Referring to the drawings, the present invention will be explained in detail in the following.

In Fig. 1, the principle of the present apparatus is shown.

A tray, in which the oocytes are lined up and arranged, having, for example, arrangements of 12 wells in a horizontal direction and 8 wells in an orthogonal direction, in total 96 wells with an uniform depth and form, can be used, however the number of wells are not limited to 96 wells. The amphibian oocytes have generally a heavier weight in the vegetal hemisphere than in the animal hemisphere. Consequently, by designing a diameter of well in the tray to be slightly larger than that of the oocytes in use, about 80% of the oocytes in average can be maintained to keep their animal hemispheres upside, without changing the directions of oocytes arranged on the tray by rotating the cells, and the injection probability to the identical hemispherical surface can be increased up without using any other special means. A form of a well for arranging oocytes is preferably a cylindrical form, which has a circular flat basal plane with a constant cross section parallel to the base plane from the base plane to the open end aperture of the above well, or a form with a conical base. A diameter of the open end of the above well should be larger than that of the amphibian oocytes in use. In addition, due to the above-mentioned reason, it is preferable to keep a space for rotating the oocytes in a well in which physiological saline is filled, and specifically the fact that a maximum diameter of well set as 105 - 150% of the diameter of oocytes in use is preferable, has been confirmed experimentally. For example, since the diameter of *Xenopus* oocytes is approximately 1.3 mm, it becomes possible to fix the oocytes in a specified direction without damaging the oocytes by designing the diameter of a well as about 1.4 - 2 mm. Preferable form of a well in the present invention is, for example, as shown in Fig. 2, a conical shaped well with an angle of base at 90° , diameter 1.4 mm and depth in cylindrical part 0.56 mm. Furthermore, by using a syringe in



addition to an use of the above tray, it is possible to operate with an increased injection probability into the same surface sides of different oocytes or it is possible to operate so that the specified surface, for example, the vegetal hemispheres of the oocytes are directed upward.

Examples of samples to be injected include, but are not limited to gene, pigment, protein, peptide, and drug. In the example herein below, oocytes, into which histamine receptor cRNA is injected, will be described, but an injectable gene is not limited to cRNA and can be DNA, RNA and synthetic oligonucleotides. A needle for injecting a sample is preferably a pipette-like needle, but is not limited. In order to obtain the cell information such as positional direction of oocytes, visual information through the digital camera 8 is applied. Further, a means for detecting the contact of the injection needle 6 with the surface of oocyte is exemplified as the visual information through CCD camera 7. However, a means required for obtaining the information is not limited to the digital camera 8 and CCD camera 7. For example, a sensor, which can detect changes of pressure, temperature, voltage, moisture or pH, may be mounted on the injection apparatus, and the surface of oocytes can be detected based on these informations.

The oocytes before injection of sample are arranged in the wells on the tray 9, and the physiological saline 14 is filled in the tray 9, then the tray is set on the orthogonal moving table 11 and the horizontal moving table 12. It is preferable to determine the position of oocyte 13, to which the sample is injected from the injection needle 6, by controlling movement of the orthogonal moving table 11 and the horizontal moving table 12 to the direction for X-axis and the direction for Y-axis with the control unit 1. Contrary to the constitution in Fig. 1, however, the constitution can be applied by fixing the tray 9 with a movable injection needle 6.

When the tray 9 is located at a position of broken line, the oocyte may be photographed by the digital camera 8, and to transfer the image data to the control unit 1, and the information of quality and positional direction of oocytes can be accumulated.

The horizontal moving table 12 and the orthogonal moving table 11 are operated by an indication of the control unit 1, and the center of the first oocyte 13, which is located

in the defined position in the oocytes arranged on the tray 9, moves to the downward position under the position of the gene injection needle 6. At this point, the injection needle moving table 4 is operated by an indication of the control unit 1 for moving the injection needle to the direction of Z-axis, a tip of the injection needle 6 mounted on the injection unit 5 moves downward to the position slightly distant from the surface of the oocyte, for example, close to the front by several hundred mm. At this point, by observing the image taken by CCD camera 7 in the monitor 3, indication is given from the auxiliary control unit 2, and the injection needle moving table 4, operated to descending direction slowly. The contact of the tip of the injection needle 6 with the surface of the oocyte 13 can be detected by visual information, pressure changes, temperature changes, electric changes, moisture changes, or pH changes, then the injection needle moving table 4 is stopped at this position. This position is a reference point for the subsequent gene injection operation. This point is made to memorize in the control unit 1 and the following operation is performed. Namely, moving distance and depth of the injection needle for the vertical direction against a plane of the tray from the above reference point to the position of injecting sample are set, and the injection needle 6 is stuck at the setting depth to inject the previously fixed amount of the sample. For the injection of sample, a control for Z-axis direction can be performed, for example, such like that the injection needle moves downwardly to 0.2 mm from the contact point of the injection needle 6 on the surface of oocyte 13. The optimum injection depth of the injection needle 6 into the oocyte is different depending on the type of sample to be injected and the object for injection and can be set freely. The sample can not diffuse into the oocyte, if the injection depth is too shallow, and if it is too deep, the probability for damaging a nucleus and a oocyte is increased. Consequently, it is preferable to inject a sample at the almost constant depth from the standpoint of expression efficiency. For example, in the case that mRNA is injected into the cytoplasm in order to express a protein, sticking the needle to the depth of 0.02 - 0.1 mm from the cell surface is preferable. On the other hand, in the case that DNA is injected into a nucleus in order to express a protein, sticking the needle to the depth of 0.05 - 0.2 mm from the cell surface is preferable on animal hemisphere. However, since

the form of oocyte may be deformed at the injection, actually the sample is injected at the shallower position than the predetermined depth. Time for injecting the sample is controlled by setting time for inserting the needle in the oocyte depending on the amount of sample to be injected. In order to improve injection efficiency, a plurality of the injection needle 6 can be used. In this case, the movement of the relative position between the injection needle and the tray by the indication from the driving unit of the apparatus in one-dimensional direction or two-dimensional direction may be sufficient.

Subsequently, the sample is automatically injected at the indicated time, rate and injection depth into the desired numbers of other oocytes in the tray 9 by automatic control. Further, since size of oocytes may have some deviation, a function for detecting a position of the surface in each time of injection can be applied. In addition, the information of oocyte condition is memorized in the computer and is able to output on demand.

The system can be made so that the movement of the injection needle 6 and the oocyte 13 in the injection or the visual information of oocyte at the injection is memorized in the computer and after termination of injection operation, position and depth of sample injection, and characteristics of oocytes can be read out. In this case, the visual information of each oocyte is preferably related with the position on the tray by numbering the oocytes, for example.

The amphibian oocytes are known to exist the animal hemisphere and the vegetal hemisphere, and each hemisphere has different function. We have found that in case that mRNA was injected into the oocyte, functional expression efficiency of protein was different in each case of injecting mRNA into the animal hemisphere and the vegetal hemisphere. In case of injecting histamine receptor mRNA, the expression efficiency is higher in the injection into the animal hemisphere than the vegetal hemisphere, as a result the oocyte with large ligand response can be obtained. On the other hand, in case that protein with fluorescent protein, or gene there of and pigment are injected, information on color and light with higher sensitivity can be obtained by the injection into the vegetal hemisphere. As described above, injection of sample into the animal hemisphere can be made for about 80% of oocytes on the tray by using the above tray. In case that the oocytes,

to which sample is injected into the specific surface of oocytes such as only for the animal hemisphere or the vegetal hemisphere, are expected to be obtained, as described above, each oocyte is treated for arranging upward direction of the specific hemisphere by using syringe before injection of sample. Alternatively, the area information for sample injection on the surface of oocytes is obtained by the detection means for the visual information or by the black and white discrimination sensor in the injection of sample, and as a result of the thus obtained information, the oocyte, to which the sample is injected into only the surface of interest directed for upward direction, can be collected. As a result, the oocytes having substantially identical condition in the injected position can be obtained. In the present invention, the "specific area" means the position including the animal hemisphere, the vegetal hemisphere or the equatorial area of the oocytes.

By using of the apparatus having the above constitution, the sample can be injected into the specific area and depth of the amphibian oocytes, and the oocytes which have almost same quality of the expression efficiency of the injected sample (injection efficiency), can be produced rapidly in mass production. Accordingly, the present invention provides a method for injecting a sample to the specific position and depth of the amphibian oocytes by using apparatus of the present invention.

It is found that the sample injection efficiency can be improved by using the apparatus of the present invention as follows. Namely, in case of beginners, who have no experience for sample injection by manual operation, about 30 minutes are required for injecting samples into 25 cells, and the injection efficiency is about 30% in case of the expression rate using a sample of gene. On the contrary, using the apparatus of the present invention, time for injecting samples into 25 cells requires only 3 minutes, and the injection efficiency reaches about 80%. In case of the sample injection performed by the experts with manual operation, almost no shortage of time for injection is observed, but the injection efficiency can be improved up to 90% by using the apparatus of the present invention as compared with efficiency of 80% in the manual operation.

Consequently, as a result of using the apparatus and method of the present invention, about 80 - 90% efficiencies can be achieved without depending upon the skill of

the operators, and to sell or assign the plurality of oocytes with controlled condition of sample injection can be possibly achieved by the present invention.

Therefore, in the other aspect, the present invention provides the amphibian oocytes with guaranteed injection of the sample into the specific position and depth.

Further, the oocytes, to which the sample is injected in the specific area and depth, can be collected, sold or assigned. In the occasion of sale or assignment, a plurality of oocytes are packaged, and a label 22, in which the information on the type of sample, the injection date, the term for guarantee of quality, setting condition such as the position and the depth of sample injection, and guaranteed injection efficiency are described, can be attached (Fig. 3).

According to the present invention, the oocytes can be sold or assigned with the information on the specific depth and area of the injection, and on the expression of protein encoded by the injected gene. In the event for selling or assigning the guaranteed oocytes regarding efficiency of sample injection or efficiency of expression, the efficiency of sample injection can be guaranteed, for example, by co-injecting a sample with protein containing pigment or chromophore, fluorescent protein, or gene encoding these proteins, counting numbers of oocytes emitting color or fluorescence, and indicating the ratio as an indicator of efficiency of sample injection. Although the coinjection can be performed in the mixed form, in case that both the sample to be coinjected and protein for detection are injected in the form of gene, a gene coding fused protein can be used.

Referring to Fig. 4, an example for calculating the efficiency of sample injection or efficiency of expression by using fluorescence from co-injected fluorescent protein as an indicator, will be explained. In the present example, a case that rate of injection of histamine receptor gene is determined by using expression of the green fluorescent protein derived from *Aequorea forbesiana* (GFP) as an indicator, is explained. However, the injected samples are not limited to genes. Further, substances used for indicator of efficiency of sample injection are not limited to GFP.

A mixture of histamine receptor gene and green fluorescent protein gene is injected into the oocyte by using the apparatus assembled with the above constitution or

the above principles. After 24 hours from gene injection manipulation, light of wavelength at 488 nm is irradiated to the oocytes, and then the expressed green fluorescent protein emits fluorescence of 507 nm. Oocytes with emitted fluorescence of 507 nm are classified into "light" and oocytes without emitted fluorescence is classified into "dark". Fig. 4 shows that these oocytes are stimulated with histamine and are classified by the presence or absence of response. As shown in Fig. 4, in the "light" oocytes, 85% of oocytes (34 out of 40 cells) respond to histamine. Namely, histamine receptor gene can be injected in more than 85% of the oocytes. On the contrary, in the "dark" oocytes, 90% or more of oocytes can not respond to histamine (27 out of 28 cells). Namely, histamine receptor gene can not be injected in 90% or more of oocytes. Accordingly, it is demonstrated that frequency of injection of histamine receptor gene is high in the oocytes expressing green fluorescent protein.

As clearly demonstrated by the above fact, when the objective sample is co-injected with the fluorescent protein, the efficiency of injection of the objective sample can be calculated by existence of fluorescence as the indicator, as a result, sale or assignment of oocytes with guaranteed efficiency of sample injection is possible.

Next, a means for production, sale or assignment of oocytes obtained by the present invention for the specific use, which is exemplified by using oocytes injected with human histamine receptor cRNA, will be described. However, gene for use of injection is not limited to cRNA, and DNA, RNA and oligonucleotide can be used.

The histamine receptor cRNA is injected into oocyte by using apparatus and method of the present invention. In this case, when means for obtaining visual information such as sensor for discriminating black and white color or CCD camera/digital camera is used, the oocytes to which cRNA is injected in the animal hemisphere, and the oocytes to which cRNA is injected in the vegetal hemisphere can be differentiated.

After gene injection, histamine receptor is expressed in the oocytes within 24 hours. After passing 24 hours from histamine receptor gene injection, membrane potential of the oocytes, in which histamine receptor may be expressed, is held at -60mV by two electrodes voltage clamp method. Under such conditions, addition of sample containing

histamine to the oocytes results in interaction between histamines in the sample and histamine receptors, and the signal transduction system in the oocyte is activated to generate ionic current, then the electric response of oocyte against histamine can be shown. The oocytes after 24 hours are stimulated with  $1 \times 10^{-6}$  M histamine and its electric current response is measured. Fig. 5 shows comparison of electric current response of the oocytes, to which gene is injected in the animal hemisphere and in the vegetal hemisphere. As shown in Fig. 5, differences between electric current responses against histamine depending on the injected site of the gene are observed. Namely, the oocytes with good ligand response can be obtained in the case that gene is injected in the animal hemisphere rather than in the case that gene is injected in the vegetal hemisphere.

Consequently, for example, the oocytes group, to which histamine receptor cRNA is injected only in the animal hemisphere, can be used as good sensor with high sensitivity for histamine. Further, according to the present invention, sale or assignment of oocytes, to which the sample is injected into the specific position and depth, for the purpose of using them as sensors can be made. As easily recognized by the person skilled in the art, examples of sample to be injected for use of oocytes obtained by the present invention as sensors, are genes encoding receptors for any ligands other than for histamine, antibodies having reactivity for specific antigens, and glycoproteins having specific sugar chain, but are not limited.

Histamine receptor gene 31 is injected into the oocyte 13 by using the apparatus assembled with the above constitution or using the above principles. In Fig. 6, the injection into the vegetal hemisphere is shown. After injection of histamine receptor gene, histamine receptor 32 is expressed in the oocytes 13 within 24 hours. As the same as in the above, after passing 24 hours from histamine receptor gene injection, membrane potential of the oocytes, in which histamine receptor 32 may be expressed, is held at -60mV by two electrodes voltage clamp method. Under such conditions, addition of sample 33 containing histamine to the oocytes 13 results in interaction between histamines in the sample and histamine receptors, and the signal transduction system in the oocyte is activated to generate ionic current, then the electric response 34 of oocyte 13 against histamine can be

shown. In case that sample 35 without histamine is added, since no substance, which interacts with the receptor, exists, the oocyte 13 can not respond to histamine 36.

The oocyte expressed histamine receptor response against histamine containing samples can be used as the indicator. Since mass production of oocytes having identical condition for injection of the sample can be possible by using the apparatus for sample injection of the present invention, the amphibian oocytes can be used for screening of the ligand or antigen reacting with receptor or antibody. The screening can be performed by using plurality of oocytes, in which sample such as gene is injected under the substantially equal condition and protein or other substances is expressed, and comparing the result of reactions of oocytes with different ligands.

Further in the other use of the present invention, for example, the expressed protein can be extracted by crushing the oocytes in which protein is expressed. The protein and other products can be effectively extracted, for example, by controlling the condition for injection with using apparatus of the present invention, and by using oocytes, to which sample is injected into the animal hemisphere.

A method for transporting the oocyte according to the present invention, to which the sample is injected, will be described referring to Fig. 6. In case of sale and transport of the above oocytes, the oocytes 13 are put into the vessel 21, to which the solution of buffer conventionally used for the amphibian oocytes dissolved with antibiotics such as gentamicin sulfate, penicillin and streptomycin is filled. The vessel 21 is packaged using packaging material such as styrene form, and is preferably transported by maintaining temperature at 4 - 25°, more preferably at 18 - 22° by using cold insulator 23 while avoiding shock, as shown in Fig. 3.

The composition of the solution is not limited, and the composition of the following can be used preferably. The solution PH is adjusted to 7.5.

NaCl	96 mM
KCl	2 mM
CaCl <sub>2</sub>	1.8 mM
MgCl <sub>2</sub>	1 mM



HEPES	5 mM
Gentamicin sulfate	50 µg/ml
Sodium pyruvate	2.5 mM
Penicillin	10 U/ml
Streptomycin	10 µg/ml

Examples of preferable vessel for sale and transport are not limited. Preferably, vessels are those in which oocytes can move relatively freely inside with cap freely opened and closed, and the above solution is preferably filled to about 95% of the vessel volume. For example, in case of conical tube 50 ml, preferably about 100 - 200 oocytes, more preferably about 130 - 180 oocytes are contained. This ratio corresponds to about 0.3 - 0.5 ml/oocyte, but is varied depending on types of oocytes and types of vessels.

As shown in Fig. 7, the oocyte 13, to which sample have been injected, is recovered from the tray 9 by using syringe 41. In this time, based on the recorded information, any one of oocytes, to which sample is injected into the animal hemisphere or the vegetal hemisphere, can be recovered. The recovered oocytes are transferred into the vessel 21. Buffer solution for Amphibia 42 is filled in the said vessel 21, and the solution is exchanged for several times. After exchanging, buffer solution for Amphibia 42 is filled in the the said vessel 21 again, and preferable amount of antibiotics 43 is added thereto. The cover of tube is closed and put into the outer case 44 with cold insulator. The outer case 44 is filled with packaging material 45, fixed the vessel 21 and transported to the customer by conventional transporting means.

By this method, the vessel can be transported to the customer without damaging the function of oocytes to which the sample is injected.

In the occasion of sale or assignment, the information including date of injection, place of injection of sample, condition for injection such as depth, recovery rate and term for guarantee of quality is provided. For that purpose, for example, paper describing such information may be attached or label 22 may be attached to the vessel 21 containing the oocytes (Fig. 3).

In case of injecting gene, about 24 hours is required for expression, and life-span

of the oocytes is about 7 days after injection, preferable for safety about 5 days. Consequently it is preferable to describe date and time of injection and, corresponding to that, a description indicating that “best use before X X (day - month)” in order to clearly indicate effective term after injection.

As for the injection site of the oocyte, sample can be injected to the animal hemisphere in about 80% of oocytes by using the above tray, and it is also possible to improve expression efficiency based on the accumulated information in the apparatus as well as using co-expression using the present apparatus.

#### Effect of the Invention

According to the present invention, the sample can be injected into the oocyte of Amphibia such as frog with a constant depth precisely, and mass production of oocytes having an identical quality such as efficiency of injection can be rapidly performed. Quality of an oocyte or area of needle injection can be recorded as the information.

Further, the oocytes obtained by the method of the present invention and into which the sample is injected in the specified position and depth, can be recovered, and the efficiency of injection of sample is guaranteed for the purpose of sale or assignment. In addition, selling or assigning of the oocytes can be made by specifying the use depending on the type of the injected sample.